Effects of α-amylase, amyloglucosidase, and their mixture on hierarchical porosity of rice starch

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Direct hydrolysis of native starch at sub-gelatinization temperatures is financially desirable because it reduces the costs associated with the high temperatures required for gelatinization. Generally, enzymes can erode the entire granule surface or sections of it (exocorrosion) or digest channels from selected points on the surface towards the center of the granule (endocorrosion). This behavior occurs because the amorphous region is relatively exposed and accessible at the surface of the granules. α- amylases (AM) are endo-amylases that randomly cleave internal α(1,4) glucosidic bonds of the polysaccharide chains and β-amylases are exo-amylases, which cleave α(1,4) glucosidic bonds from the non-reducing ends of the starch chain. Glucoamylases (amyloglucosidases, AMG) are exo-amylases that can act on both α(1,4) and α(1,6) glucosidic bonds from the non-reducing ends of the starch chain and isoamylases are debranching enzymes that hydrolyze exclusively α(1,6) glucosidic bonds, leaving long linear polysaccharides. The utilization of a mixture of AM and AMG with reaction times ranging from 12–30 h showed increased starch hydrolysis efficiency of cassava, corn, mung bean, sweet potato, and sago native starch, due to a synergetic action of enzymes. In this study, waxy native rice starch was enzymatically treated at a sub-gelatinization temperature, applying reaction times of 3, 6, and 12 h, respectively. This work aimed to investigate the effect of AM, AMG, and their mixture (AM+AMG) on the morphology and hierarchical porosity of the obtained porous starches.

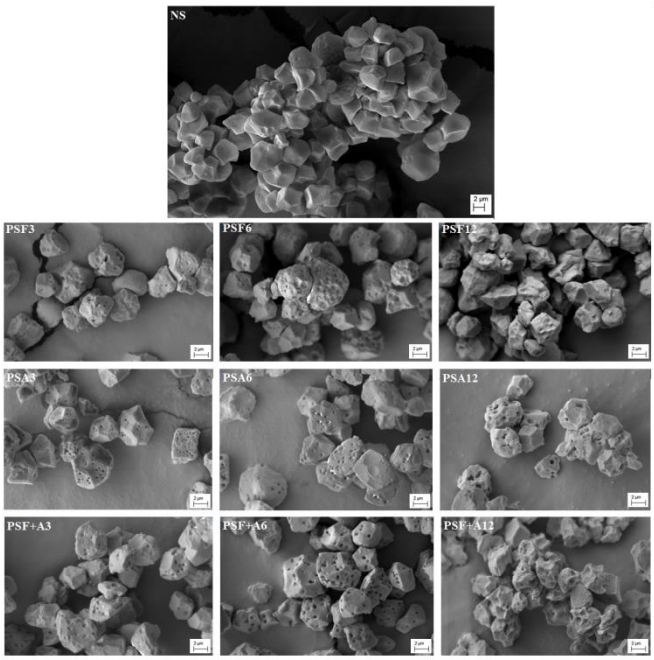

Figure 1. *SEM micrographs of waxy native rice starch (Native) and porous starches (AM3, AM6, AM12, AMG3, AMG6, AMG12, AM+AMG3, AM+AMG6 and AM+AMG12).*

The degree and type of porosity can be controlled using adequate reaction conditions. The reaction time of 12 h was excessive, resulting in the collapse of the formed pores that weakens the granule structure. The reaction time of 6 h was found to be appropriate for the three enzymatic treatments studied, optimizing the usual enzymatic reaction time.

Table 1. *Feret diameter, pore size and methylene blue adsorption of waxy native rice and porous starches.*

	Scanning electron microscopy	Specific surface area	Adsorption capacity
	Feret diameter (μm)	Pore size (Å)	Methylene blue adsorption (%)
Native	-	12.91 ± 0.03i	52.7 ± 0.31d
AM3	0.28 ± 0.09a	61.86 ± 0.07f	91.7 ± 0.42a
AM6	0.29 ± 0.08a	61.89 ± 0.08f	92.5 ± 0.57a
AM12	0.33 ± 0.08a	68.34 ± 0.03c	90.5 ± 0.55ab
AMG3	0.22 ± 0.04a	58.95 ± 0.04g	87.2 ± 0.27c
AMG6	0.23 ± 0.04a	64.34 ± 0.04e	86.2 ± 0.89c
AMG12	0.29 ± 0.10a	72.35 ± 0.04a	84.9 ± 0.25c
AM+AMG3	0.31 ± 0.05a	45.15 ± 0.03h	87.1 ± 0.74c
AM+AMG6	0.34 ± 0.05a	65.71 ± 0.06d	88.1 ± 0.94bc
AM+AMG12	0.45 ± 0.10a	69.98 ± 0.06b	85.3 ± 0.46c

The techniques used in the characterization of the porous starches showed a porosity distribution for all samples, and it was possible to identify the presence of macropores (SEM), mesopores (porosimetry of nitrogen) and micropores (adsorption capacity). The addition of AM suggested the formation of an increased micropores fraction, whereas the AM+AMG mixture contributed to more mesopores and macropores fractions.

Dura, A., Rosell, C. M., Physico-chemical properties of corn starch modified with cyclodextrin glycosyltransferase. *Int J Biol Macromol* 2016, *87*, 466-472.
Benavent-Gil, Y., Rosell, C. M., Comparison of porous starches obtained from different enzyme types and levels. *Carbohydr Polym* 2017, *157*, 533-540.
Guo, L., *et al.*, The physical and adsorption properties of different modified corn starches. *Starch - Stärke* 2015, *67*, 237-246.
Gao, F.,*et al.*, Adhikari, B., Preparation and characterization of starch crosslinked with sodium trimetaphosphate and hydrolyzed by enzymes. *Carbohydr Polym* 2014, *103*, 310-318.
Malucelli, L. C., *et al.*, Porous waxy maize starch. *J Therm Anal Calorim* 2015, *120*, 525-532.
Yussof, N. S., *et al.*, Hydrolysis of native and cross-linked corn, tapioca, and sweet potato starches at sub-gelatinization temperature using a mixture of amylolytic enzymes. *Starch - Stärke* 2013, *65*, 285-295.